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Diversity in membrane composition is associated with variation in thermoregulatory capacity in hymenopterans.

Enrique Rodríguez[1], Jean-Michel Weber, Charles-A. Darveau*

Department of Biology, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

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ABSTRACT

Thermoregulatory capacity varies widely among bees and wasps, but the cellular physiology required to support such thermogenic ability remains unclear. Studies conducted on ectothermic species living in varying temperature show that cellular membrane composition is adjusted to remain functional, a process named homeoviscous adaptation. We show that the fatty acid composition of flight muscle membranes varies with thermogenic capacity in species of bees and wasps. The relative abundance of palmitate (16:0) and linoleate (18:2) decreased, while oleate (18:1) increased with increasing thoracic temperature. Species selected for the study varied over ten-fold in body mass, which in turn affected species thoracic temperature and their fatty acids profile. Nevertheless, all analyses conducted show that thoracic temperature is the main driver of flight muscle membrane composition in hymenopterans with diverse thermoregulatory capacity. These findings are in line with the predictions based on the homeoviscous adaptation hypothesis and further show that thermogenic strategy used by insect species impacts cellular membrane composition.

1. Introduction

Insects are widely distributed around the world, inhabit almost every environment, and also show remarkable diversity in thermal properties. Many are ectotherms poïkilotherms, as their internal temperature varies, but they are unable to generate and maintain enough heat to increase their body temperature above that of the environment. Therefore, environmental temperature constrains their daily activities. Other insect species are heterotherms, thus switching periodically between ectothermy and endothermy. Thermoregulation is strongly linked to the evolution of high metabolic rate associated with insect flight (Heinrich, 1981, 1995), and bee and wasp species of the Hymenoptera order display important interspecific and intraspecific variation in endothermy and thermoregulatory capacity (Stone, 1994; Stone and Willmer, 1989; Willmer and Stone, 2004). Within a species, high-altitude hymenopteran populations can be more endothermic than populations at sea level and are consequently able to forage at lower ambient temperatures, while temperate populations with poor thermoregulatory capacities rely on behavioral strategies, such as long periods of basking, to warm up (Herrera, 1995; Stone, 1993). Interspecific differences in endothermic heat generation are also found in honeybees or wasps and are thought to be linked to differences in foraging and nesting behavior, but also in morphological and physiological features of workers (Dyer and Seeley, 1987; Kovac et al., 2009).

Hymenopterans warm up for flight through different mechanisms, from basking to shivering thermogenesis, or a mix of both. During the latter, muscles do not operate myogenically as during flight. The opposite sets of muscles are declutched from the wings and contract simultaneously in a tetanus (Esch and Goller, 1991). These tetanic contractions of the dorsoventral and dorsal longitudinal indirect flight muscles generate sufficient heat during pre-flight warm-up, elevating thoracic temperature above the environmental temperature (Willmer and Stone, 2004). Overheating is avoided via enhanced convective heat loss (shunting of the haemolymph to the abdomen) or behaviorally by searching for shade, restraining activity, or by dissipating excess heat through evaporation (Heinrich, 1995). Hymenopterans thus differ in their ability to thermoregulate, but the implications of such variation on cellular properties associated with variable thermal regime remains unexplored.

Cellular membranes are sensitive to temperature, and poikilothermic organisms ranging from bacteria to plants and animals can adjust their composition to maintain homeostasis in a process named homeoviscous adaptation (HVA) (Hazel and Williams, 1990; Sinensky, 1974). The cold-induced ordering of the membrane is commonly countered by an increase in the relative abundance of unsaturated fatty acids, causing disorder to maintain membrane fluidity (Logue et al.,

* Corresponding author at: Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario K1N 6N5, Canada.
E-mail address: cdarveau@uottawa.ca (C.-A. Darveau).
[1] Present address: Laboratoire de Physiologie Animale Intégrative, Département de Biologie, Université du Québec à Rimouski, Rimouski, QC, Canada G5L 3A1.

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2000). The two fatty acid chains constitute the hydrophobic part of phospholipids which are, along with other lipid classes and various proteins, the backbone of cellular membranes. Fatty acids (FAs) are carboxylic acids with a long hydrocarbon tail, and can be either saturated or unsaturated. Saturated FAs (SFAs) only possess single bonds and allow rotation around the carbon-carbon bond, while unsaturated FAs can have one (monounsaturated, MUFAs) or two to six double bonds (polyunsaturated, PUFAs). Double bonds constrain the rotation around the consecutive carbons, and provide a kink in the chain that can affect the surrounding lipids in the bilayer (Hulbert and Else, 1999). This variability in FA composition affects the local membrane fluidity and the function of various membrane-associated structures and processes such as various oxidative enzymes (Guo et al., 2005; Miyasaka et al., 1996; Power and Newsholme, 1997), ATPases (Swanson et al., 1989; Turner et al., 2005), hormone receptors (Corcoran et al., 2007) and ion channels (Leaf et al., 2005).

Membrane FA composition has been linked with habitat-specific temperatures, as species of vertebrates living in cold climates generally possess more unsaturated membranes than species found in warmer environments (Logue et al., 2000). Moreover, this remodeling of cellular membranes with temperature acclimation has been established in a wide variety of organisms (Ayala-Del-Río et al., 2010; Uemura et al., 1995). In teleost fish, a heat stress can affect the regulation of genes linked to FA metabolism (Buckley, 2006), and membrane saturation varies with season (Guderley, 2004; Kraffe et al., 2007). In reptiles, membranes are also subject to changes in polyunsaturation such as in cold-acclimated crocodiles (Seebacher et al., 2009). Fruit flies (*Drosophila melanogaster*) subjected to rapid cold hardening, were found to respond by reductions in membrane SFAs and MUFAs, and increases in PUFAs (Overgaard et al., 2005). An appropriate phospholipid FA composition of membranes appears to be crucial to survival at freezing temperatures in many organisms such as the freeze-tolerant earthworm *Dendrobaena octaedra* (Bindesbol et al., 2009). Globally, it appears that the cellular thermal environment greatly influences membrane composition in a variety of organisms.

This study investigates how the diversity in thermoregulatory capacity found in hymenopterans affects the membrane phospholipid composition of flight muscle tissue. We tested relationships between thoracic temperature and the thoracic FA composition in temperate bee and wasp species. We expected to find higher proportions of PUFAs at lower thoracic temperature, in line with the predictions from the HVA hypothesis, while muscles operating at higher temperature should contain more MUFAs and SFAs.

2. Materials and methods

2.1. Sampling of hymenopterans and temperature measurements

Individuals from various species of hymenopterans were sampled during the spring and summer at various locations in the Ottawa area. Two superfamilies were targeted, Apoidea-Anthophila and Vespoidea. A total of 109 individuals were sampled, pertaining to 21 species and two different superfamilies of the Hymenoptera order. For one species, *Bombus bimaculatus,* both workers and queens were sampled and treated as separate data point; all analyses were also performed without the queens and results were essentially the same. Body masses of the collected specimens ranged from 27.5 to 550 mg. Immediately after capture individuals were transferred to a restraining device (50 mL syringe with netting at the open end) to immobilize them, and their thoracic surface temperature (T_{th}) was measured in under a minute. Thermograms were taken in the shade using an infrared camera (EX300, FLIR systems). Individuals were then transported in 50 mL transparent tubes, placed on ice in a cooler until arrival at the lab where they were stored in the freezer at − 20 °C. Within 24 to 48 h, specimens were identified to the species level (when not possible, to the subgenus level) using various keys (Buck et al., 2008; Michener, 2007); their body parts separated, weighted and stored in 1.5 mL CryoVials at − 80 °C. Thermographic data was stored on a computer before further analysis and extraction of the T_{th}. An Enviro-meter (Fisher scientific) was used to measure the ambient air temperature (T_a). The thorax temperature excess, the difference between thoracic surface and ambient air temperature (T_{th}-T_a) was also used to assess the endothermic capacity (Kovac and Stabentheiner, 2012). For all analyses, results for thoracic temperature excess and T_{th} were essentially the same, thus only results for T_{th} are reported.

2.2. Fatty acid composition of membrane phospholipids

Total lipids from flight muscle were extracted by homogenizing the thorax (Polytron, Kinematica, Luzern, Switzerland) in 2:1 chloroform:methanol (v/v) (Folch et al., 1957) and completing 3 cycles of shaking, centrifugation (10 min, 2000 g), and filtration. Before the last cycle, 0.25% KCl was added to help removing aqueous contaminants. The aqueous phase was discarded, and the organic phase was dried on a rotating evaporator (Büchi Rotavapor, Flawil, Switzerland). Phospholipids were separated by resuspending total lipids in chloroform before loading on solid-phase extraction columns (Supelclean 1 mL, 100 mg LC-NH2; Sigma-Aldrich; St. Louis, USA). Neutral lipids, non-esterified fatty acids, and phospholipids were separated by sequential elution using solvents of increasing polarity: isopropyl ether:acetic acid (98:2 v/v), chloroform:isopropanol (3:2 v/v), and methanol (Maillet and Weber, 2006). The FA composition of membrane phospholipids was measured after acid transesterification in 1 M acetyl chloride and methanol (90 °C for 2 h). FA methyl esters were analyzed on an Agilent Technologies 6890 N gas chromatograph (Mississauga, Ontario, Canada) equipped with a flame-ionization detector and a fused silica capillary column (Supelco DB-23, 60 m, 0.25 mm i.d., 0.25 µm film thickness; Sigma-Aldrich) using published procedures (Magnoni and Weber, 2007). Individual FAs were identified by determining exact retention time with authentic standards (Supelco, Bellefonte, PA, USA). Only the fatty acids accounting for > 1% of total FAs in membrane phospholipids are reported.

2.3. Statistical analysis

All statistical analyses were performed using the Systat 13 software. All values are presented as mean ± SE. Dependent and independent variables were first tested for normality using the Shapiro-Wilk test. Relationships between body mass and FA relative abundance in birds and mammals follow power functions (Hulbert et al., 2007). Therefore, variables were log-transformed to linearize the data. Nevertheless, we also tested several non-linear regression models, but the data fit was marginally improved in only a few cases and subsequent results were essentially the same. Normality of residuals was also verified using the Shapiro-Wilk test, and homoscedasticity was assessed using Levene's test.

We first tested the effect of body mass on T_{th}, taking superfamily as a covariate using an analysis of covariance (ANCOVA). For each FA, we first tested the relationships with each independent variable: body mass or thoracic temperature (T_{th}), taking superfamily into account using ANCOVAs. We further tested the effect of body mass, thoracic temperature and superfamily combined. For all analyses, interactions were tested and only reported when significant. Finally, we present correlations between thoracic temperature and membrane composition independent of body mass, by performing analyses on the residuals obtained from significant regressions with body mass.

3. Results

3.1. Thoracic temperature variation with body mass

Thoracic temperature (T_{th}) increased with increasing body mass of